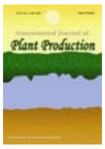

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Germination pattern of naturally grown *Lathyrus* and *Vicia* species to different methods and seedbeds

U. Basaran[a,*], H. Mut[a], O. Onal Asci[b], I. Ayan[c], Z. Acar[c]

[a]*Bozok University, Faculty of Agriculture, Department of Field Crops, Turkey.*
[b]*Ordu University Faculty of Agriculture, Department of Field Crops, Turkey.*
[c]*Ondokuz Mayıs University, Faculty of Agriculture, Department of Field Crops, Turkey.*
*Corresponding author. E-mail: ugur.basaran@bozok.edu.tr

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Abstract

In many forage legumes, hard seed coat is the main problem impeding the germination by preventing water and gases transport in seed. We evaluated the effects of mechanical scarification, chilling, different seedbeds (peat and sand) and their combination on the germination ratio and time of 9 naturally grown legume species belonging *Lathyrus* and *Vicia* genus. Control seeds showed very low germination ranging from 0% in *V. narbonensis* and *V. sativa* to 24% in *L. annuus* among to species. Mechanical scarification highly increased germination in all the species ranging from 27% in *V. sativa* to 96% in *L. sphaericus*. The highest germination of all the investigated species (ranging between 100 and 51%) was determined in the combination of mechanical scarification and sowing in peat treatments. Under this treatment, germination was improved greatly from 1% in control to 100% in *V. bythinica* while the germination of *V. narbonensis* increased from 0% in control to 51%. Any treatment which is highly promotes germination of one species generally accelerated the germination time of same species. The effect of treatments subjected to present study on germination ratio and time was generally, with exceptions higher for *Lathyrus* species compare to *Vicia* species.

Keywords: Germination; *Lathyrus*; *Vicia*; Hard seed coat; Scarification; Peat.

Introduction

Vicia and *Lathyrus* belongs to *Leguminosae* are two large genera with 150 species in *Vicia* (Kupicha, 1976) and 160 species in *Lathyrus* (Alkin et al., 1986). Many species of *Vicia* and *Lathyrus* genera provide excellent forage and crop legumes. Moreover, these genera are thought to possess potential for further development as important new forage sources (Fu et al., 1996; Yakupoglu et al., 2011).

The Mediterranean region, and in particular Turkey, is the centre of diversity of many temperate leguminous crops (Maxted et al., 1990), many of currently in use or having agricultural potential (Cocks, 1993). In Turkey, 58 *Lathyrus* and 59 *Vicia* species are naturally grown (Davis, 1970), *Vicia* species are the most cultivated annual forage legumes. *Lathyrus* species were generally grown for seed used in animal feeding and lesser human consumption (Basaran et al., 2010).

The most important germination problem of forage legumes results from very hard seed coat (Balouchi and Sanavy, 2006) defined by as seeds which remain hard at the end of the prescribed test period due to an impermeable seed coat to water and gases. In a natural setting, seed dormancy is ecologically significant. Because it delays germination until conditions are right for the establishment of seedlings and also it allows for the maintenance of an annual seed bank (Eldredge, 2007). However, from an agronomic point of view, it can be useful to overcome seed dormancy in forage legumes used for forage production, pasture improvement by over seeding and in artificial rangeland. Since the presence of hard seeds impedes rapid, uniform and high germination as required for cultivation (Plitmann and Kislev, 1989). Hence, great efforts are being made in forage legumes for breaking of hard seed dormancy.

Dormancy is a complex interaction between external environmental (Eslami et al., 2010) and inherent internal germination barriers that must be broken if germination is to occur (Baskin and Baskin, 1998). Hard seeds can be softened artificially by different treatments such as mechanical or acid scarification, cooling at very low temperatures in moist or dry conditions (Pritchard et al., 1988). Also storage regimes (wet-cold, dry-cold and dry-warm) and periods effects the seed dormancy (Esmaeili et al., 2009). It is reported that wild *Lathyrus* species, promising for improving new varieties, showed very low germination, however, their germination highly increased compare to control with mechanical scarification at different levels among species (Ayan et al., 2005; Acar and Basaran, 2007). Olivensia and Devasa, (1997) reported that germination increased with sulphuric acid application in 14 *Vicia* taxon and, germination after scarification fit an exponential curve in most taxa, expect *V. lutea* spp. *vestita*.

In most legume species physical dormancy is broken by some environmental factors in the field condition, but it is not clear that how this mechanism works (Wen et al., 2009). On the other hand the effect of each method on germination rate depends on the plant species and varieties due to different seed coat structure (De Sousa and Filho, 2001), thus it can be suggested that the most effective method in breaking the dormancy of forage legumes should be determined by the application of each method with different period, concentration or degree combinations to the seeds in order to obtain maximum germination rate (Balouchi and Sanavy, 2006). Although, in many previous studies, significant effects of different physical, chemical treatments and heat application on breaking hard seed dormancy have been displayed in various species, the effect of seedbed on physical dormancy has not been sufficiently studied. There are very few study on dormancy pattern of naturally grown *Lathyrus* and *Vicia* species. Therefore, the objectives of this study were to determine the effects of mechanical scarification, chilling and different seedbeds such as peat and sand on breaking dormancy and germination pattern of naturally grown *Lathyrus* and *Vicia* species.

Materials and Methods

Naturally grown 5 *Lathyrus* and 4 *Vicia* species were evaluated in this study (Table 1). They were chosen due to their potential for forage breeding with advantageous yield and growth habits (Yakupoglu et al., 2011).

Table 1. Names, life forms and 1000 seed weights of 9 studied legumes.

Species	Life-form	1000 seed weight (g)
L. ochrus L. (DC)	Annual	97.67
L. hirsutus L.	"	25.42
L. aphaca L. var *affinis*	"	13.82
L. sphaericus L.	"	16.52
L. annuus L.	"	67.70
V. sativa L.	"	78.45
V. lutea L.	"	76.73
V. bythinica L.	"	56.05
V. narbonensis L.	"	121.16

Seed samples were collected from around Samsun located Black Sea Region of Turkey and lies coastal area. Original seeds were sown in experiment field of Agricultural Faculty of Ondokuz Mayıs University (41^o 21' 51" N, 36^o 11' 27" E) in November 2006. Harvested seeds of each species from the experiment were stored for six months at room temperature (18±2 ^{o}C) in dark condition with 60-65% relative humidity and then subjected to germination analysis under natural light.

As main treatments, mechanical scarification (S), chilling (CL) and their combination (S+CL) was evaluated. Also scarificated and untreated seeds were sowed in different seedbed; peat (P), sand (SD), scarification+sowing in peat (S+P) and scarification+sowing in sand (S+SD).

Mechanical scarification is very difficult with equal effect on all species due to different seed size (Table 1) and coat thickness. For this reason, coat of each seed was niced with surgery lancet for scarification treatment to guarantee water and gasses transport (being carefully to avoid embryo damage).

Chilling effects on germination was evaluated by exposing the seeds wet condition at 5 ^{o}C (Bewley and Black, 1994) during a week, than seeds were subjected to germination test in petri dishes. For scarification+chilling (S+CL) firstly, seeds were firstly scarificated then exposed chilling. For control (C), scarification (S), chilling (CL) and combination scarification and chilling (S+CL), seeds were placed in petri dishes on the filter paper and moistened with sterile water.

Scarificated and untreated seeds were sown in peat and washed sea sand to 1 cm depth. Peat and sand was filled in multi-celled trays, having 50 cells with 3 cm depth and 3 cm diameter. Each seed was sown in one cell. Peath (Klasman^R); Ec: 40 mS/M (± 25%), PH; 5.5-6.5, NPK (14.16.18)/15kg/m^3.

All germination tests were conducted at 18±2 ^{o}C and germinated seeds were counted on day 7, 14 and 21. The emergence of the radicle considered as germination (Zhang and Mu, 2009). The germination percentage in days 7, 14 and 21 was calculated by following formula:

[Germination (%) = No. of germinated seed (between days 0-7, 7-14, 14-21)/ no. of total seed x 100]

Three replicates of 50 seeds were used for each treatments and species. The results were evaluated individually for each species. The effects of treatments on germination of each species were evaluated by using means of replications.

Results and Discussion

Germination ratio and time in all the species were affected by the treatments (Figures 1 and 2) Control seeds showed very low germination ranging from 0 to 24% among species. Scarification (S), sowing in peat (P), sowing in sand (SD) and S+P, S+SD combinations increased germination in all the species in comparison with control (C).

Effects of chilling (CL) on germination varied depending on the species (Figure 1). The CL decreased germination in *L. annuus* and *V. lutea* and not effected in *V. narbonensis* and *V. sativa*. However, although to a lesser degree, CL increased seed germination of *L. aphaca*, *L. hirsutus*, *L. ochrus*, *L. sphaericus* and *V. bythinica* compared to control. Cold stratification or prechilling stimulates cold winter conditions in seeds with internal dormancy (Young and Young, 1986). Van Ashcce et al. (2003) reported that lower temperature (5 ^{o}C) was optimum compared to higher (30 ^{o}C) temperature for germination of *V. aphaca*. The cold stratification period necessary to break dormancy may last from a few days to several months, depending upon the species (Bonner et al., 1974). So, the different response of the species to the CL is probably a result of their individual needs for cooling temperature or period.

Mechanical scarification (S) highly increased germination of all the species (Figure 1). It was observed that all scarificated seeds absorbed the water and their swelling. *L. sphaericus* (96%), *L. aphaca* (88%) and *L. hirsutus* (82%) reached up to high germination ratio with mechanical scarification. As reported in many study, reason of dormancy in legumes generally results from hard an impermeable seed coat, and it can be broken by thinning or breaking the seed coat (Tarawali et al., 1995). It is reported that mechanical scarification was very effective to eliminate seed dormancy in naturally grown *Trifolium* and *Medicago* species (Uzun and Aydin, 2004; Onal Asci et al., 2011). In general, *Lathyrus* species had higher germination ratio (58-96%) than *Vicia* species (27-60%). These results in the present study clearly suggested that all the species studied had a high percentage of impermeable seed. However, especially *Vicia* species and *L. ochrus* may have other dormancy mechanism besides impermeable seed coat. Germination ratio in these species was not higher than 60% although all the seeds swell in 24 hours after scarification.

The chilling combined with mechanical scarification (S+CL) increased germination of the species compared to control except *L. annuus*, however, it dramatically decreased seed germination in all species except *L. aphaca* compared to alone S treatments. For instance seed germination of *L. annuus* (63%) under alone S decreased and it was 13% when S+CL applied. This result suggested that alone S is more effective than S+CL on germination of the species studied. Several reports have shown that the intact seed coat retards water uptake and hence protects against imbibitions injury (Duke et al., 1986; McDonald et al., 1988). Damaging the seed coat decreases the barrier to rapid water uptake and increases the susceptibility to cooling (Tully et al., 1981; Taylor and Dickson, 1987).

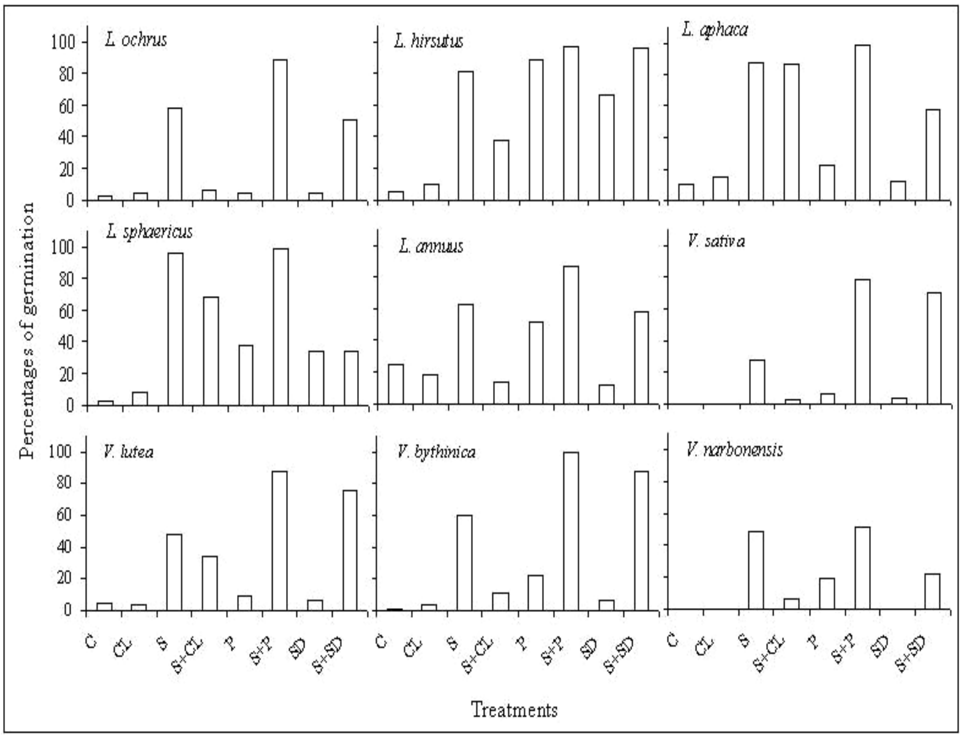

Figure 1. Germination persentages for 9 species end of the days 21 in rospense to following treatments: control (C), scarification (S), chilling (CL), scarification+chilling (S+CL), sowing in peat (P), scarification+sowing in peat (S+P), sowing in sand (SD), scarification+sowing in sand (S+SD).

As a seedbed treatment, sowing in path (P) alone increased the germination of *L. hirsutus* (89%), *L. annuus* (52%), *L. sphaericus* (38%) compared to control. Moreover, germination of *L. hirsutus* seed applied P was higher than seeds scarificated (82%). This result is very interesting. Because all the species had seed coat dormancy and, peat can not have mechanically disturb the seed coat. In this case, permeability of seed coat may have resulted from microbial activities or chemical subsistence in peat. Peat enhances microbial activities and, includes high organic matter, humic acid, fulvic asid and nitrate which are stimulate seed germination (Baskin and Baskin, 1998; Piccolo et al., 1993). The other seedbed treatment, sowing in sand (SD) also increased seed germination of *L. hirsutus* (66%), *L. sphaericus* (34%). But germination of *L. annuus* decreased in compared to control treatments.

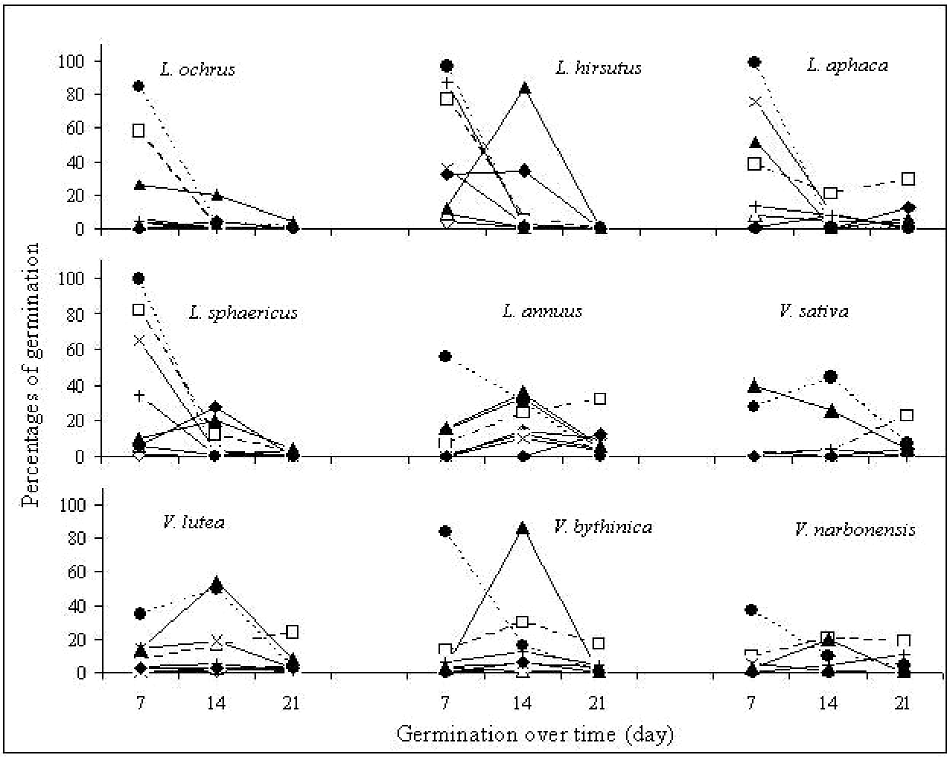

Figure 2. The germination over time of 9 species in response to treatments. ◊: control (C), □: scarification (S), Δ: chilling (CL), X: scarification+chilling (S+CL), +: sowing in peat (P), ●: scarification+sowing in peat (S+P), ♦: sowing in sand (SD), ▲: scarification+sowing in sand (S+SD).

The combination of mechanical scarification and sowing in peat (S+P) also significantly increased seed germination in all species. Germination in 8 of 9 species applied S+P was higher than 80%, furthermore, this range for four species *V. bythinica*, *L. aphaca*, *L. sphaericus* and *L. hirsutus* was 97-100%. When S and P treatments are combined (S+P), all the investigated species better germinated in compare to treatments of S and P alone and others. So, S+P treatment was the most effective method in breaking dormancy for two genus of *Lathyrus* and *Vicia*. Similarly S treatment combined with SD (S+SD) promoted seed germination but a lesser degree than S+P treatment. S+SD treatment highly increased especially the germination of *L. hirsutus* (96%), *V. bythinica* (88%) and *V. lutea* (76%). Germination condition was highly effective on both breaking of dormancy and general germination pattern of the species studied. Therefore the indigenous dormancy in these species may be removed by proper germination condition. Germination was higher and rapid when scarificated seeds sown in peat compare to alone S or S+SD treatments (Figures 1 and 2).

The effect of the treatments on germination time was variable (Figure 2). As seen in figure 2, the effect of treatments on germination time depended on the species. In addition, any treatment which is highly promotes germination of one species generally accelerated the germination time of same species. S+P treatment highly accelerated germination time compared to other treatments. Moreover germination showed a peak in first 7 days for most of the species that was being used for their treatment of S+P. And also, under S+P treatment, high portion of the total germination for 21 days occurred in first 7 days in the species those are *L. ochrus*, *L. hirsutus*, *L. aphaca*, *L. sphaericus*, *V. bythinica* and *V. norbonensis* (Figure 2). Differently, in *V. sativa*, the highest germination ratio in first 7 days was optained from seeds which used for S+SD treatment. Alone scarificated seeds accessed maximum germination percentage in longer time than seeds applied S+P combination. Beter germination in the treatment of S was due to air conditions and better nutritional conditions. The effects of the alone P treatment on germination time was greatly higher in *L. hirsutus* with up to 80% germination in first 7 days. The S+CL treatment highly accelerated the germination time in *L. aphaca*, *L. sphaericus*, *L. hirsutus* in compare to other species. Although S+SD treatment promoted total germination of all species, it was not highly accelerate germination time except *V. sativa* (Figure 2). This result showed that the conditions in sand not good as in peat for germination of the legume species studied. In general, germination of the species of two genus reached the high amount of their total germination at the end of the day 14. However, with the exception of *L. ochrus* and *L. hirsutus*, germination of the species was slow and continued during 21 days when alone S treatment was applied, although their seeds swell in first 24 hours. This result clearly indicated that rapid water uptake is not mean of rapid germination; water absorption must be controlled after the breaking of seed coat for the rapid germination. For this reason, one of the important effects of the seedbed on germination may be controlling water uptake of the seed, besides nutrition content and aeration capacity.

Conclusion

One of the major problems in Forage legume is hard seed content which results in poor stand establishment because of low and non-homogeneous seedling emergence. This study showed that naturally grown *Lathyrus* and *Vicia* species have seed coat dormancy which can easily broken by scarification. However, especially *Vicia* species showed also physiological dormancy. The effect of seedbed was significant and, some species reach to high percentage of germination when their seed were only sown in peat. The effect of the treatments changed depend on the species, however, the highest total germination in all the species was determined under combination of scarification and sowing in peat. In addition scarification+sowing in peat greatly accelerate germination time in most of the species.

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